PULSE ELECTRIC FIELD EFFECT ON CERVICAL CANCER CELL: A PLANT BASED ANTI-CANCER STUDY

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For my father Ganeson Sengan, my mother Saraswathy Arjunan, my siblings Hemaraaj Ganeson, Devaraaj Ganeson, Thurgashini Ganeson, my husband Dayamugil Ponnudurai, my uncle Vasantha Rajan and my aunt Khaitiri Devi.

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ABSTRACT

Cancer is listed in top ten global causes of death in 2016. The percentage of cancer incidence and mortality are steadily rising over the year. This necessitates alternate anti-cancer treatment as the conventional treatment have resulted in high number of long- term survivors who are left to deal with the aftereffect of their therapy. Drug delivery is a challenging aspect in medical community as the plasma membrane is non-permeable to external molecules. From this context, a study was proposed to use of natural plant extract Artocarpus altilis which will be uploaded into cervical cancer cell with the aid of electrical pulses or electroporation technique to investigate on the anti-cancer mechanism. Electroporation parameters utilized in this study ranging from 200V/cm, 400V/cm, 600V/cm, 800V/cm, 1000V/cm of pulse amplitude, while pulse duration ranging from 100µs, 500µs, 1ms, 5ms and 10ms with single pulse. Three parts of artocarpus altilis tested in this research study which is the peel, pulp and whole fruit. The concentration of all three parts which were tested on cervical cancer cell ranged from 10µg/ml, 20µg/ml, 30µg/ml, 40 µg/ml, and 50µg/ml with methanol as the solvent. It is found that pulse electric field increased the cell spreading up to 129.8% with 600V/cm of pulse amplitude, which stimulates to the highest cell length percentage. Apart from that, cell viability found to be decrease with an increase in pulse amplitude and pulse duration. 600V/cm of pulse amplitude and 10ms of pulse duration stimulates highest cell viability with 53.3% as compared to control group (without treatment). Cell viability of cervical cancer cell measured when cell subjected to electrical field alone, extract alone and both electrical field and extract and the result of the study revealed that lowest cell viability stimulated by the combination of electrical pulse and extract up to 28% of cell viability. Thus the outcome of this research revealed new targets and electrochemical pathways for cervical cancer treatment while with the discovery health of patients will be maintained and there will be an improvement in general life expectancy.

ABSTRAK

Kanser disenaraikan dalam sepuluh penyebab utama kematian pada tahun 2016. Peratusan kejadian penyakit kanser dan kematian terus meningkat sepanjang tahun. Maka rawatan alternatif anti-kanser diperlukan kerana rawatan konvensional telah menyebabkan banyak kesan sampingan pada jangka masa yang panjang. Sistem penyampaian ubat merupakan aspek yang mencabar dalam bidang perubatan kerana membran plasma tidak menerima molekul luar. Dalam konteks ini, satu kajian dicadangkan untuk menggunakan ekstrak tumbuhan semulajadi Sukun dan bantuan medan elektrik denyut atau teknik electroporasi untuk menyiasat mekanisma anti kanser keatas sel kanser serviks. Parameter elektroporasi yang digunakan dalam kajian ini terdiri daripada 200V/cm, 400V/cm, 600V/cm, 800V/cm, 1000V/cm amplitud denyut, manakala tempoh denyut adalah antara 100µs, 500µs, 1ms, 5ms dan 10ms. Tiga bahagian Sukun diuji dalam kajian penyelidikan ini iaitu kulit, pulpa dan buah tumbuhan tersebut. Konsentrasi ketiga-tiga bahagian yang diuji pada sel kanser serviks antara 10µg/ml, 20µg/ml, 30µg/ml, 40µg/ml, dan 50µg/ml dengan metanol sebagai pelarut. Adalah didapati bahawa medan elektrik denyut meningkatkan panjang sel sehingga 129.8% dengan amplitud nadi 600V/cm, yang merangsang peratusan panjang sel tertinggi. Selain itu, daya tahan sel didapati berkurangan dengan peningkatan amplitud nadi dan tempoh nadi. Amplitud denyut 600V/cm dan 10ms tempoh denyut merangsang daya maju sel terendah dengan peratusan sebanyak 53.3% berbanding dengan kumpulan sel kawalan. Daya tahan sel sel kanser serviks diukur apabila sel tertakluk kepada medan elektrik sahaja, ekstrak sahaja dan kedua-dua medan elektrik dan ekstrak dan hasil kajian mendedahkan bahawa daya tahan sel paling rendah dirangsang oleh gabungan nadi elektrik dan ekstrak sehingga 28% daripada daya tahan sel. Oleh itu, hasil kajian ini menunjukan sasaran baru elektrokimia untuk rawatan kanser serviks malah kesihatan pesakit akan terjamin.

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LIST OF SYMBOLS AND ABBREVIATIONS

Cm	Centimetre
E	Electric field strength
ml	Mililitre
ms	Milisecond
nm	Nanometre
T	Pulse duration
V	Voltage
µL	Microlitre
µs	Microsecond
CO_2	Carbon dioxide
°C	Degree celsius
DNA	Deoxyribonucleic matrix
ECM	Extracellular matrix
EF	Electrofusion
EG	Electrogenetheraphy
EP	Electroporation
ECT	Electrochemotherapy
FBS	Fetal bovine serum
HELA	Cervical cancer cell
IC_{50}	Half maximal inhibitory concentration
NEP	Non-electroporation
PBS	Phosphate bovine serum
RPMI	Roswell Park Memorial Institute Media
TES	Tryple express solution

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LIST OF ASSOCIATED PUBLICATIONS

Journals

1. Ganeson, S., Jamil, M. M. A., Adon, M. N., Wahab, R. A., & Ahmad, M. S. (2017). Artocarpus altilis compound based study for anti-cancer proliferation on HeLa cell via Electroporation Method. *Journal of Fundamental and Applied Sciences*, 9(4S), 310-318. **[ISI]**
2. Jamil, M. M. A., Ganeson, S., Mammam, H. B. & Wahab, R. A. (2018). Artocarpus altilis extract effect on cervical cancer cells. *Materials Today: Proceedings*, 5(7), 15559-15566. **[Scopus]**
3. Ganeson, S., Jamil, M. M. A., Mamman, H. B., & Rahman, N. A. A. (2018). Correlation between electrical field strength and pulse width analysis on cell viability. In *Journal of Physics: Conference Series*, 1019(1), 012012. IOP Publishing. **[ISI & Scopus]**

Conference proceedings

1. Ganeson, S., Ambar, R., & Jamil, M. M. A. Influence of artocarpus altilis fruit extract on tumour cell. *2018 9th IEEE Control and System Graduate Research Colloquium (ICSGRC 2018).* **[Submitted]**
2. Mahadi, M., Jamil, A., Mamman, H. B., & Ganeson, S. (2018, April). Investigation of Anti-Cancer Proliferation Properties of Luffa Acutangula on Ht29 Using Electroporation Method. In Proceedings of the 2018 8th International Conference on Biomedical Engineering and Technology (pp. 108-111). ACM.

Lecture Notes (Electrical Engineering LNEE-Springer)

1. Ganeson, S., Jamil, M. M. A., & Adon, M. N. Electrical pulsed artocarpus altilis on cervical cancer cell. *10th International Conference on Robotics, Vision, Signal Processing & Power Applications (ROVISP2018).* **[Submitted]**
2. Ganeson, S., Ambar, R., & Jamil, M. M. A. Breadfruit peel extract impact on hela cancer cell viability and proliferation. *10th International Conference on Robotics, Vision, Signal Processing & Power Applications (ROVISP2018).* **[Submitted]**

LIST OF COPYRIGHTS, AWARD AND SCHOLARSHIP

Copyrights

1. Cervical cancer cell inhibition via electroporation technique.
   Registration Number: (LY2017005064) 2017.
2. Artocarpus altilis extract study on cervical cancer cell for anti-cancer treatment.
   Registration number: (LY2017005066) 2017.

Award

1. **Faculty Level: Second Runner Up** in 3 Minutes Idea Presentation on *4 December 2016*, Faculty of Electrical and Electronic Engineering.

Scholarship

1. Ministry of Higher Education, Malaysia. MyBrain15 (MyMaster).

International Product Innovation

1. Suhassni Ganeson, Muhammad Mahadi Abdul Jamil "Investigation of Electroporation Method and Extract on Cell Cervical Cancer Cell" Indonesia-Malaysia-Thailand Symposium on Innovation and Creativity (iMIT SIC 2018) Riau, Indonesia (August 2018). [Silver Medal]
2. Suhassni Ganeson, Muhammad Mahadi bin Abdul Jamil,Radzi bin Ambar, Ridhwan Abdul Wahab, Md Asri Ngadi, Johan Mohamad Sharif "Investigation of Electroporation Method and Extract on Cell Cervical Cancer

Cell" International Eureka Innovation Exhibition (i-EIE 2018) UniKL Kedah (October 2018). [Bronze Medal]

CHAPTER 1

INTRODUCTION

1.1 Background of study

The number of cancer disease occurrences and mortality is expected to increase rapidly due to population growth, aging factor, and unhealthy lifestyle that increase the risk of cancer. Cancer is referred to an abnormal cells which replicates in uncontrolled manner in the body, accumulated different mutations and it occurs when the body's natural control mechanism fails to operate (Susan, 2010). Cancer can develop anywhere in the body and can be categorized based on either biological type or the initial site; part where the cancer originated. There are five major categories of cancer; sarcomas (develops in the bone, fat, muscles, cartilage, connective tissues), carcinoma (cancer that originated in the skin and tissues), leukemia (cancer that begins in blood-forming tissue), lymphomas (cancer that starts begins in the immune system) and myeloma (begins in the cell of immune system; bone marrow).

To date there are many treatments available in order to help the cancer patients, for an instance surgery, immune therapy, radiation therapy, hyperthermia and so on. Electrochemotherapy, the combination of electroporation and impermeant anti-cancer drugs, is one of the effective anti-cancer treatments. For a better understanding, it is an application of electroporation (short and intense electric pulses) which increases cell membrane permeability, and enhance the uptake of anti-cancer drugs to treat the tumorous cells. Cisplatin and bleomycin have proven to be the most appropriate drugs to be use with electroporation due to the chemical properties.

However, advances in cancer treatment have resulted in high number of long- term survivors who were left to deal with the aftereffect of their treatments. Drugs usage in cancer therapy leads to multiple permanent side effects. The use of bleomycin drugs associated with fatal side effects like induction of pulmonary toxicity. Utilization of bleomycin, will cause pulmonary syndromes such as bronchiolitis obliterans, eosinophilic hypersensitivity and interstitial pneumonitis (Sleijfer, 2001). On the other hand, neurotoxicity, nephrotoxicity, ototoxicity, nausea and vomiting are the side effects of cisplatin drug. Moreover this type of drugs has the potential in interrupting treatments with effectiveness reduction and affect patient's quality of life (Santabarbara, Maione, Rossi, & Gridelli, 2016). Apart from anti-cancer drugs, patients undergoing electrochemotherapy felt of discomfort due to muscle contraction during to the high frequency electric pulses delivery (Mamman, Jamil, & Adon, 2017).

There are an increasing number of new promising studies of anti-cancer using natural plants as chemotherapeutic agent. Natural plants that contain anti-cancer properties like Curcumin and Artocarpus altilis wood which inhibit proliferation in breast cancer cell. Apart from that, jackfruits have also shown anti-cancer properties against laryngeal, cervical, breast and lung cancer. Moreover, bioactive compounds are found in various types of berries and saffron proved to have chemo-preventive properties. Recent studies claimed that the combination of turmeric, ginger and garlic established to oppose the growth of breast cancer cell.

This study focuses on Artocarpus altilis to investigate the anti-proliferation on cervical cancer cell. Artocarpus altilis is an extensively grown and nutritious tree fruit. It is also known as breadfruit while *Sukun* in Malaysia and Artocarpus altilis scientifically. Each of every parts of Artocarpus altilis has its own importance and it is widely distributed in tropical and subtropical regions including Malaysia and Indonesia (Fajriah, Mozef, Artanti, Lotulung, & Abbas, 2013). There are numbers of studies focusing on Artocarpus altilis, and proven to have several interesting biological properties including anti-cancer, antioxidant, antibacterial, anti-inflammatory, antihypertensive and many more (Fajriah et al., 2013; Vasugi, Sudhahar, & Anandajogopal, 2012). As the use of drugs in cancer therapies, comes with multiple side effects, Artocarpus altilis has been chosen for this study taking into consideration its anti-cancer properties to inhibit cancer cell proliferation.

Since the Artocarpus altilis extract is impermeant, or in another word unable

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